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3 AN ASSEMBLED HEMATIN, METHOD FOR FORMING SAME AND METHOD FOR  
4 POLYMERIZING AROMATIC MONOMERS USING SAME

5  
6 CROSS-REFERENCE TO RELATED APPLICATIONS

7 This is a continuation-in-part of U.S. Application Serial No.  
8 09/994,998, filed November 27, 2001, in the names of Sukaut  
9 Tripathy, et al, which, in turn, claims the benefit of U.S.  
10 Provisional Application No. 60/253,109, filed November 27, 2000,  
11 both of which are hereby incorporated herein by reference.

12  
13 STATEMENT OF GOVERNMENT INTEREST

14 The invention described herein may be manufactured and used  
15 by the U.S. Government for governmental purposes without the  
16 payment of any royalty thereon.

17  
18 BACKGROUND OF THE INVENTION

19 Recently, there has been an increased interest in tailored  
20 development of polyaromatic polymers, particularly polyaromatic  
21 polymers that are electrically conductive and/or have useful  
22 optical properties. Examples of electrically conductive polymers  
23 include certain polyanilines, polythiophenes, polypyrroles, and  
24 polyphenols. These conductive polyaromatic polymers may be used  
25 in a variety of electronic devices, including electro-chromic

1 devices, light-emitting diodes, electrostatic discharge  
2 protection, and light weight batteries. Of these polyaromatic  
3 polymers, polyanilines are the most extensively studied, due  
4 largely to superior electrical properties, such as high discharge  
5 capacity.

6 In addition to the above-named electrical properties, thermal  
7 and structural properties of polyphenols have long been exploited.  
8 In particular, phenol-formaldehyde resins, such as novolacs and  
9 resols, have found wide application as wood composites, laminates,  
10 foundry resins, abrasives, friction and molding materials,  
11 coatings and adhesives, fiber binders, and flame retardants. The  
12 use of formaldehyde in polyphenol synthesis, however, presents a  
13 significant toxicological and environmental hazard.

14 Despite the industrial utility of polyaromatic polymers,  
15 their synthesis remains problematic. Known difficulties in the  
16 synthesis of such polymers include inconsistent product  
17 composition, due in part to extensive branching of the polymers.  
18 In addition, many of the polyaromatic polymers are insoluble, or  
19 sparingly soluble, in common solvents, leading to poor  
20 processability. The use of toxic reagents, as noted above, is  
21 another undesirable feature of current synthetic methods. A  
22 search for new methods of synthesizing polyaromatic polymers has  
23 not heretofore yielded a commercially viable approach.

24 Many of the synthetic approaches to forming polyaromatic  
25 polymers use a heme-containing enzyme to catalyze the

1 polymerization. Any such catalyst must necessarily be stable and  
2 active under acidic conditions, as acidic conditions are required  
3 in order to synthesize an electrically conductive form of a  
4 polyaromatic polymer, such as polyaniline.

5 An enzyme suggested for aromatic molecule polymerization is  
6 horseradish peroxidase (HRP). Unfortunately, HRP and other  
7 peroxidases are inactive at low pH and are prohibitively expensive  
8 to use commercially. Hematin has been used to mimic the catalytic  
9 activity of HRP. However, despite its lower cost, hematin is a  
10 non-ideal catalyst for commercial polymerizations because of its  
11 low solubility in acidic, aqueous media. The low solubility of  
12 hematin under these conditions leads to a low rate of  
13 polymerization and poor yields.

14 The mechanism for HRP catalyzed polymerization involves the  
15 interaction of the heme-iron cofactor of the enzyme with the  
16 peroxide, yielding an oxidized heme-iron complex. Subsequently,  
17 the oxidized heme-iron complex reacts with the substrate in a one-  
18 electron transfer reaction to produce the substrate radical and a  
19 new iron-heme complex followed by the coupling of the radicals to  
20 form the polymer.

21 This enzymatic approach has not been extended to  
22 polythiophenes or polypyrroles, which have high electrical  
23 conductivity. This is because monomers, such as (3,4)-  
24 ethylenedioxythiophene (EDOT) and pyrrole (PYR), complexed with  
25 the active site of the enzyme catalyst, cause deactivation of the

1       latter and have proved to be unsuitable substrates for this  
2       enzymatic polymerization. This deactivation phenomenon  
3       drastically limits the prospects for the enzymatic synthesis of a  
4       wide range of polymers for possible industrial applications. The  
5       present invention evolved from exploration of the possibility of  
6       usage of the hydroxy ferriprotoporphyrin Hematin to serve as a  
7       catalytic center.

8               There is a need for a low cost, high efficiency means of  
9       synthesizing polyaromatic electronic and photonic polymers, which  
10      means is compatible with conditions required to synthesize  
11      polymers with commercially desirable properties.

#### 12 13                               SUMMARY OF THE INVENTION

14              Accordingly, an object of the invention is to provide a  
15      derivatized hematin suitable for use as an enzyme catalyst in  
16      polymerization of monomers, a method for forming assembled and  
17      derivatized hematins, and to methods for polymerizing aromatic  
18      monomers with an assembled or derivatized hematin.

19              Another object of the invention is to provide hematin  
20      derivatized with one or more non-proteinaceous amphipathic groups,  
21      particularly wherein the amphipathic group is polyethylene glycol.

22              A further object of the invention is to provide a method for  
23      preparing a derivatized hematin by reacting hematin with an  
24      amphipathic compound, wherein the hematin is derivatized with an  
25      amphipathic compound in the presence of a carboxylic acid

1       activating compound for an aprotic base, particularly wherein the  
2       amphipathic compound is soluble over a pH range from about pH 1 to  
3       about pH 12.

4             A still further object of the invention is to provide an  
5       assembled hematin which includes alternating layers of hematin and  
6       a polyelectrolyte on an electrically charged substrate,  
7       particularly wherein the polyelectrolyte is cationic.

8             A still further object of the invention is to provide a  
9       method of forming assembled hematin by alternately depositing one  
10      or more layers of hematin and one or more layers of a  
11      polyelectrolyte on an electrically charged substrate.

12            A still further object of the invention is to provide a  
13      method for polymerizing aromatic monomers, such as anilines or  
14      phenols, more particularly, wherein the polymerization takes place  
15      in the presence of a template, preferably anionic.

16            Still another object of the invention is to provide a method  
17      for polymerizing aromatic monomers by contacting an aromatic  
18      monomer and a template with an assembled hematin. Preferably, the  
19      aromatic monomer is an aniline or a phenol.

20            A further object of the invention is to provide a method for  
21      polymerizing an aromatic monomer, which method includes combining  
22      the aromatic monomer with a derivatized hematin catalyst, wherein  
23      the hematin preferably is derivatized with polyethylene glycoat  
24      (PEG), and the derivatized hematin catalyst and the aromatic  
25      monomer are combined with a peroxide to initiate the reaction.

1           A further object of the invention is to provide a novel  
2       method for the synthesis of a conducting complex of polyaniline  
3       and multi walled carbon nano tubes (MWCNT) which results in the  
4       production of polyaniline/MWCNT which has enhanced electrical and  
5       chemical stability, and improved processability.

6           A still further object of the present invention is to provide  
7       a method as described above which results in the synthesis of a  
8       polyaniline/MWCNT polymer complex which may be used for  
9       applications including but not limited to, nanowires in  
10      microchips, high performance nanotubes; reinforced conductive  
11      composites; single-molecular transistors, electron emitters for  
12      flat panel displays, chemical sensors and artificial muscle  
13      actuators.

14          With the above and other objects in view, the present  
15      invention is directed to resolving the current limitations of  
16      catalysts used in the commercial synthesis of polyaromatic  
17      polymers, by reducing the cost of catalyst and by providing a  
18      catalyst that is active and stable over a wide range of pHs. A  
19      feature of the present invention is the provision of a method for  
20      forming derivatized hematis which are water-soluble and  
21      recyclable, virtually eliminating the need for toxic reagents and  
22      solvents, thus creating an environmentally friendly synthesis for  
23      polyaromatic polymers. Further, the derivatized hematis of the  
24      present invention, in combination with a template, reduce the

1 amount of branching during polymerization, leading to structurally  
2 more consistent product.

3 In accordance with a further feature of the invention, there  
4 is provided a method for syn-enzymatic polymerization of  
5 polypyrroll (PPYR) and/or EDOT in the presence of sulfonate  
6 polystyrene (SPS), which results in a novel complex of PPYR and/or  
7 poly (3,4)-ethylenedioxythiophene (PEDOT) with SPS, which has  
8 exceptional stability, and good processability.

9 There have been attempts to use different forms of hematin  
10 for catalysis, but it was seen that the catalytic activity was  
11 incomparably lower than that of the enzyme. It is known to  
12 provide for the efficient synthesis of polyaromatic compounds  
13 catalyzed by hematin in mixed solvent systems or buffer systems of  
14 high pH values. It has been found suitable to use a chemically  
15 modified hematin to effectively synthesize conducting polyaniline  
16 in the presence of polyelectrolyte templates. Work in this area  
17 has attempted to manipulate this artificial catalyst towards the  
18 synthesis of conducting PEDOT or PPYR, with the ultimate goal of  
19 expanding the versatility of this hydroxy ferriprotoporphyrin  
20 based catalyst. The method described herein enables the synthesis  
21 of such electroactive polymers, suitable for conductive  
22 transparent coatings.

23 In accordance with a still further feature of the invention,  
24 there is provided a unique template assisted approach for the  
25 synthesis of water-soluble polymers has been found, involving

1 enzymatic polymerization of aniline and phenol with HRP as the  
2 catalyst in the presence of an anionic polyelectrolyte. In this  
3 case, the polyelectrolyte, such as SPS serves three main  
4 functions, namely, to electrostatically align the aniline  
5 monomers to promote a para directed approach, to provide  
6 counterions for doping the polymer, and to maintain water  
7 solubility. Aside from the polyelectrolyte macromolecular  
8 templates, micellar templates like sodium dodecylbenzene sulphonic  
9 acid, and biological templates, like DNA, have been investigated  
10 and seen to be successful nano-reactors in the one-pot enzymatic  
11 synthesis of conducting polyanilines. Thus, the template provided  
12 an environment wherein the pH and the charge density near the  
13 template molecule were different from those of the bulk solution,  
14 the polymerization being carried out at pH 4.0, (peroxidases are  
15 active in the pH range of 4.0 - 8.0).

16 In accordance with another feature of the invention there is  
17 provided a novel synthesis of water soluble PEDOT and PPYR using  
18 PEG hematin as an efficient catalyst in the presence of SPS as a  
19 template. EDOT and PYR have been copolymerized using this unique  
20 catalyst.

21 In accordance with one purpose of the invention, as embodied  
22 and broadly described hereinabove, a method for a matrix assisted,  
23 syn-enzyme-catalyzed polymerization of aniline comprises the  
24 preparation of an aqueous solution containing aniline, sulfonate  
25 MWCNT, PEG-Hematin syn-enzyme and reaction initiator (hydrogen



1 peroxide). The procedure is a one-step, *in situ* reaction, which  
2 is highly selective and which produces minimal by-products and  
3 chemical waste. The resulting polymer solution can be used  
4 immediately as is or purified via such techniques as dialysis and  
5 centrifusion and for subsequent processing strategies.

6 The above and other features of the invention, including  
7 various novel details of construction and combinations of steps,  
8 will now be more particularly described with reference to the  
9 accompanying drawings and pointed out in the claims. It will be  
10 understood that the particular methods embodying the invention are  
11 described by way of illustration only and not as limitations of  
12 the invention. The principles and features of this invention may  
13 be employed in various and numerous embodiments without departing  
14 from the scope of the invention.

#### 15 16 BRIEF DESCRIPTION OF THE DRAWINGS

17 Reference is made to the accompanying drawings in which are  
18 shown illustrative embodiments of the invention, from which its  
19 novel features and advantages will be apparent.

20 In the drawings:

21 FIG. 1 shows the functionalization of hematin with  
22 polyethylene glycol (PEG) in the presence of N,N'-carbonyl  
23 diimidazole, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and  
24 dimethylformamide (DMF);

1           FIG. 2 shows the Fourier Transform Infrared (FTIR) spectra of  
2           hematin and PEG-hematin. The inset shows an expanded region  
3           between 1500 and 1700cm<sup>-1</sup>;

4           FIG. 3a shows the <sup>1</sup>H NMR spectra of hematin and PEG-hematin  
5           in DMF-d<sub>7</sub>. The inset shows the disappearance of the hematin  
6           carboxylic acid peak when it is derivatized with PEG;

7           FIG. 3b shows the <sup>1</sup>H NMR spectra of hematin and PEG-hematin  
8           in D<sub>2</sub>O;

9           FIG. 4 shows the catalytic activity of hematin and PEG-  
10          hematin for the oxidation of pyrogallol at pH 4.0;

11          FIG. 5 shows the UV-vis absorption spectrum of aniline  
12          monomers and of polyaniline formed during PEG-hematin catalyzed  
13          polymerization;

14          FIG. 6 shows the time dependent UV-vis absorption spectra of  
15          the polyaniline-sodium polystyrene sulfonate (SPS) complex formed  
16          at pH 4 over 2 hours after initiation of polymerization;

17          FIG. 7 shows the pH-dependent UV-vis absorption spectra of  
18          the polyaniline-SPS complex formed after initiation of  
19          polymerization;

20          FIG. 8 shows the UV-vis absorption spectra of polyaniline-SPS  
21          complex as it is titrated with 1 N NaOH and 1 N HCl, demonstrating  
22          that the complex can be reversibly dedoped and redoped using base  
23          or acid, respectively;

24          FIG. 9 shows a cyclic voltammogram of a solution cast film of  
25          polyaniline-SPS complex synthesized at pH 1.0;

1           FIG. 10 shows the pH-dependent UV-vis absorption spectra of  
2 polyaniline-lignin sulfonate complexes formed during  
3 polymerization;

4           FIG. 11 shows UV-vis absorption spectra of polyaniline-DNA  
5 formed during PEG-hematin catalyzed polymerization;

6           FIG. 12 shows CD spectra of polyaniline-DNA formed during  
7 PEG-hematin catalyzed polymerization;

8           FIG. 13 shows time-dependent UV-vis absorption spectra of the  
9 polymerization of 2-methoxy, 5-methylaniline catalyzed by PEG-  
10 hematin;

11           FIG. 14 shows pH-dependent UV-vis absorption spectra of  
12 polyaniline-dodecylbenzenesulfonic acid complexes formed during  
13 polymerization;

14           FIG. 15 shows UV-vis absorption spectra of a SPS-polyphenol  
15 complex formed during polymerization; and

16           FIG. 16 shows titration of polyaniline/polystyrene sulfonate  
17 from pH 4 to pH 10 and back to pH 4.

#### 18 19                           DESCRIPTION OF THE PREFERRED EMBODIMENTS

20           The present invention generally includes a derivatized and an  
21 assembled hematin, along with methods of preparing the hematins.  
22 The invention also includes methods of polymerizing aromatic  
23 monomers in a reaction catalyzed by an assembled or a derivatized  
24 hematin.

1           The methods of the present invention include the use of  
2   hematin, a hydroxyferriprotoporphyrin, which has been derivatized  
3   with one or more non-proteinaceous amphipathic groups. Examples  
4   of amphipathic groups include phosphoglycerides, sphingomyelin,  
5   glycolipids, substituted or unsubstituted polyethers and  
6   polyalkylene glycols, substituted or unsubstituted polyamines such  
7   as polyethyleneimine, polyallylamine, and poly(diallylamine);  
8   polyammonium groups, such as poly(allylammonium salts),  
9   poly(trimethylallylammonium salts), poly(triethylallylammonium  
10   salts), poly(dimethyldiallylammonium salts),  
11   poly(diethyldiallylammonium salts), and polysaccharides such as  
12   hydroxypropyl cellulose, hydroxymethyl cellulose, and hydroxyethyl  
13   cellulose.

14           Preferred amphipathic groups include polyalkylene glycols,  
15   such as polyethylene glycol and polypropylene glycol. Preferably,  
16   polyethylene glycol groups have a molecular weight of about 400 to  
17   about 100,000, or more, and preferably a molecular weight of about  
18   5,000 to about 15,000.

19           In one embodiment, the present invention is a method of  
20   derivatizing hematin, which includes reacting hematin with one or  
21   more amphipathic compounds, thereby forming a derivatized hematin.  
22   In a preferred embodiment, the hematin is reacted with one or more  
23   amphipathic compounds in the presence of a carboxylic acid  
24   activating compound and an aprotic base. In a preferred  
25   embodiment, the carboxylic acid activating compound is a

1 dialkylcarbodiimide. In another preferred embodiment, the  
2 amphipathic compound is a substituted or unsubstituted  
3 polyalkylene glycol. Preferably, the polyalkylene glycol is  
4 polyethylene glycol.

5 "Carboxylic acid activating compounds," as used in the  
6 present description, are compounds that serve to couple a  
7 nucleophile, such as a hydroxyl, amine, or thiol group, to a  
8 carboxylic acid, thereby forming an ester, an amide, or a  
9 thioester linkage. Suitable carboxylic acid activating compounds  
10 include dialkylcarbodiimides, preferably diisopropylcarbodiimide  
11 and dicyclohexylcarbodiimide; N,N'-carbonyldiimidazole;  
12 nitrophenol, preferably o-nitrophenol and p-nitrophenol;  
13 pentahalophenol, preferably pentachlorophenol, and  
14 pentabromophenol; N-hydroxysuccinimide; tosyl chloride; 1-  
15 hydroxybenzotriazole; and N-ethyl-N'-(3-dimethylaminopropyl)  
16 carbodiimide.

17 "Aprotic bases," as used herein, include bases without an  
18 exchangeable proton. Suitable aprotic bases include  
19 trialkylamines, such as trimethylamine, triethylamine,  
20 diisopropylethylamine and triphenylamine; pyridine; pyrimidine;  
21 1,8-diazabicyclo[5.4.0]undec-7-3n3 (DBU); and 1,3,5-triazine.

22 Derivatized hematins of the present invention can be  
23 prepared, for example, by reacting about one-half to about ten  
24 mole equivalents of an amphipathic compound, such as polyethylene  
25 glycol, with hematin in the presence of an excess of a carboxylic

1 acid activating compound, and an aprotic base, in an aprotic  
2 solvent such as dimethylformamide or an ether. The mixture is  
3 allowed to stir for about 6 hours to about 6 days, and is then  
4 quenched with a large volume of water or other protic solvent.  
5 The unreacted reagents are removed by extraction of the reaction  
6 mixture with an organic solvent such as ethyl acetate. The water  
7 layer is concentrated, preferably by lyophilization, to yield the  
8 derivatized hematin.

9 In another embodiment, the present invention is assembled  
10 hematin, which includes one or more layers of hematin alternating  
11 with one or more layers of a polyelectrolyte deposited on a  
12 substrate. In a preferred embodiment, polyelectrolyte is a  
13 cationic polymer, such as a poly(dialkyldiallylammonium salt) or a  
14 poly(trialkylallylammonium salt). More preferably, the  
15 polyelectrolyte is poly(dimethyldiallylammonium chloride).

16 In another embodiment, the present invention includes a  
17 method of forming assembled hematin, by alternately depositing  
18 layers of hematin and a polyelectrolyte onto an electrically  
19 charged substrate. Preferably, the polyelectrolyte is a cationic  
20 polymer, and more preferably is a poly(dialkyldiallylammonium  
21 salt) or a (trialkylallylammonium salt, such as poly  
22 (dimethyldiallylammonium chloride).

23 Assembled hematins of the present invention can be prepared,  
24 for example, by dipping a charged substrate, such as a negatively-  
25 charged hydrophilized glass slide, into about 0.1 mM to about 100

1 mM hematin having a pH from about 6 to about 12 at about 0°C to  
2 about 50°C for about 1 minute to about 100 minutes. The substrate  
3 is washed with deionized water and dried with a stream of gas,  
4 such as nitrogen or argon. The substrate with a single layer of  
5 hematin is dipped into about 0.1 mM to about 100 mM  
6 polyelectrolyte having a pH from about 6 to about 12 at about 0°C  
7 to about 50°C for about 1 minute to about 100 minutes. The  
8 substrate is washed with deionized water and dried from a stream  
9 of gas, such as nitrogen or argon. The process can then be  
10 repeated, from about 1 to about 100 times, to produce multiple  
11 alternating layers (or bilayers) of hematin and the  
12 polyelectrolyte on the substrate. For a positively-charged  
13 substrate, the order of dipping into hematin and a polyelectrolyte  
14 is reversed.

15 In another embodiment, the present invention includes a  
16 method of polymerizing an aromatic monomer to form a complex of a  
17 polymerized aromatic monomer and a template, by contacting the  
18 aromatic monomer and the template with the assembled hematin.  
19 Preferably, the template is an anionic polymer, such as  
20 poly(styrene sulfonic acid) or a salt thereof. In another  
21 preferred embodiment, the aromatic monomer is a substituted or  
22 unsubstituted aromatic compound, such as an aniline or a phenol.  
23 In yet another preferred embodiment, the complex of the  
24 polymerized aromatic monomer and the template forms in solution or  
25 the complex forms on the assembled hematin. The complex forming

on the assembled hematin can contact one or more layers of hematin or the polyelectrolyte.

Aromatic monomers include substituted and unsubstituted aromatic compounds. Suitable aromatic compounds include 4-(p-hydroxyphenylazo)pyridine and 4-(p-hydroxyphenylazo)pyridinium methiodide. Preferred aromatic compounds for polymerization include aniline, phenol, and 2-methoxy, 5-methylaniline.

Suitable substituents on aromatic monomers will not significantly reduce the rate of polymerization as compared to an unsubstituted aromatic monomer (e.g., will not reduce the rate of polymerization by more than ten-fold). Examples of suitable substituents for aromatic monomers include, for example, halogen (-Br, -Cl, -I, and -F), -OR, -CN, -NO<sub>2</sub>, -COOR, -CONRR<sub>1</sub>, -SO<sub>k</sub>R (where k is 0, 1, or 2), -NRR<sub>1</sub>, -SR, haloalkyl groups, and -NH-C(=NH)-NH<sub>2</sub>. R and R<sub>1</sub> are independently, -H, an aliphatic group, and aralkyl group, a heteroaralkyl group, and aromatic group, or a substituted aromatic group. A substituted aromatic monomer can have more than one substituent.

Polymerizations catalyzed by assembled hematins of the present invention can be carried out, for example, in a buffered solution, ranging from about pH 1 to about pH 12, at about 0°C to about 50°C. An aromatic monomer and a template are added to the buffered solution, such that the ratio of aromatic monomer to template repeat unit is about 5 to 1 to about 1 to 5. The concentration of aromatic monomer is about 0.01 M to about 1 M. A



1 quantity of assembled hematin, including about 2 to about 100  
2 bilayers of hematin and polyelectrolyte, is added to the solution.  
3 A solution of a peroxide, in an amount sufficient to polymerize  
4 the aromatic monomer, is added dropwise over about 5 minutes to  
5 about 200 minutes. The reaction is maintained for about 1 hour to  
6 about 200 hours. The progress of the reaction can be monitored  
7 spectrophotometrically.

8 A peroxide, as used in the present invention, is an organic  
9 or inorganic compound that includes a -O-O- bond, such as ROOR,  
10 where R is as defined above. Preferably, one R is hydrogen, to  
11 give ROOH. Even more preferably, the peroxide is hydrogen  
12 peroxide, HOOH.

13 Suitable substrates for assembled hematin are any solids that  
14 can maintain an electrical charge. Examples of substrates include  
15 glasses (e.g., pyrex and glass slides), plastics (e.g., poly(vinyl  
16 chloride) and poly(ethylene)), ceramics, metals, and the like.  
17 Preferred substrates are glass slides, which have been  
18 hydrophilized with an aqueous alkali solution, such as Chem-solv,  
19 under ultrasonication.

20 In a preferred embodiment of the present invention, a  
21 template is combined with the derivatized hematin, an aromatic  
22 monomer, and a peroxide, such that the aromatic monomer aligns  
23 along the template and polymerizes to form a complex including the  
24 polymerized aromatic monomer and the template. A "template," as  
25 that term is employed herein, refers to a polymer or oligomer that

1 can bind, such as ionically bind, to the aromatic monomer being  
2 polymerized.

3         Suitable template polymers include polyelectrolytes, such as  
4 an anionic polymer or a cationic polymer. Anionic polymer  
5 templates include polymers that include pendant acid functional  
6 groups such as poly(vinylbenzoic acid) and salts thereof,  
7 poly(vinyl polyphosphonic acid) and salts thereof, poly(glutamic  
8 acid) and salts thereof, poly(aspartic acid) and salts thereof,  
9 poly(acrylic acid), and poly(maleic acid co-olefin) and salts  
10 thereof. Co-olefins that can be polymerized with maleic acid to  
11 form poly(maleic acid co-olefin) include 1-propene, 1-butene, 1-  
12 pentene, 1-hexene, 1-heptene, 1-octene, 1-nonene, and 1-decene.  
13 Preferred anionic polymer templates include poly(styrene sulfonic  
14 acid) and salts thereof, lignin sulfonic acid and salts thereof,  
15 and dodecylbenzene sulfonic acid and salts thereof.

16         Optically active templates can be employed in the  
17 polymerization method of the invention. When an optically active  
18 template is employed, the template can induce macro-asymmetry in  
19 the polymerized aromatic monomer due to the close association of  
20 the template with the polymerized aromatic monomer in the complex.  
21 Examples of optically active templates include polynucleic acids  
22 and salts thereof, such as rubonucleic acids and 2'-  
23 deoxyribonucleic acids. Other suitable templates include  
24 biological receptors, peptides, proteins, zeolites, caged

1 compounds, phenol red, azo compounds, azo polymers, and  
2 dendrimers.

3 In a preferred embodiment, the complex of a polymerized  
4 aromatic monomer and a template is a water-soluble complex of a  
5 polyaniline and a template. Preferably, the polyaniline (pani) is  
6 of the electrically-conducting emeraldine salt form. Emeraldine  
7 is an electrically-conducting form of pani, and has a  
8 characteristic green color when protonated, or doped.

9 In another preferred embodiment, the complex including a  
10 polymerized aromatic monomer and a template is a water-soluble  
11 complex of a polyphenol and a template.

12 In still another preferred embodiment, a polymerized aromatic  
13 monomer complexed to an optically active template has a macro-  
14 asymmetry.

15 A complex of a polymerized aromatic monomer and a template is  
16 prepared by contacting an aromatic monomer, such as an aniline or  
17 a phenol, and a template with a derivatized hematin in a solution  
18 of a pH from about 0 to about 12. Preferably, the solution is  
19 buffered, and the pH ranges from about 0 to about 7, and more  
20 preferably ranges from about pH 0 to about pH 4. The ratio of  
21 aromatic monomer to template (measured as the concentration of  
22 template repeat units) can vary from 5:1 to 1:5 (aromatic monomer  
23 : template repeat unit), and is preferably from about 2:1 to about  
24 1:2, and is even more preferably about 1:1. A catalytic amount of  
25 the derivatized hematin can be added to the reaction mixture

1        either before or after addition of the aromatic monomer. A  
2        catalytic amount of the derivatized hematin is typically between  
3        about one unit/mL and five units/mL, where one unit will form 1.0  
4        mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.

5        Preferably, the derivatized hematin is added to the solution  
6        after addition of the template and aromatic monomer. In a  
7        preferred embodiment, a peroxide is also added to the reaction  
8        mixture. The peroxide is added incrementally, such as not to de-  
9        activate the derivatized hematin catalyst, until an amount  
10       approximately stoichiometric with the amount of aromatic monomer  
11       has been added. The reaction can be monitored spectroscopically.

12       The above polymerization can be carried out in polar solvents  
13       such as ethanol, methanol, isopropanol, dimethylformamide,  
14       dioxane, acetonitrile, and diethyl ether, but is preferably  
15       carried out in water.

16       The functionalities of the polymers may be tuned to impart  
17       requisites, such as sensing, electrochemical, optical and  
18       electronic properties through copolymerization with functionalized  
19       monomers. The polymers have sites for further modifications, such  
20       as covalently coupling other functionalities and even biomolecules  
21       through simple coupling chemistry.

22       The conducting polymers in these polymer complexes allows for  
23       use in a wide range of applications including, but not limited to,  
24       chemical and biological sensing, electrostatic shielding,  
25       corrosion protection, rechargeable batteries, flexible light-

1 emitting diodes, electrochromic devices, smart windows, chaff  
2 materials, electromagnetic radiation absorbers and modulators, and  
3 drug delivery systems.

4 Accordingly, to achieve the foregoing objects and in  
5 accordance with the purpose of the invention, as embodied and  
6 broadly described herein, a method for matrix assisted, syn-  
7 enzyme-catalyzed polymerization or copolymerization of PYR, PEDOT  
8 and aniline comprises the preparation of an aqueous solution  
9 containing PYR and/or PEDOT, SPS, Hem-PEG syn-enzyme and a  
10 reaction initiator (hydrogen peroxide). The procedure is a one-  
11 step, in situ reaction, which is highly selective and which  
12 produces minimal by-products and chemical waste. The resulting  
13 polymers or copolymers solution can be used immediately as is or  
14 purified via such techniques as dialysis and centrifugation.

15 Matrix materials may include, but are not limited to,  
16 electrolytes which have various aromatic backbones and/or pendant  
17 groups, aliphatic backbones and/or pendant groups, optically  
18 active (chromophoric) backbones and/or pendant groups,  
19 electrically active backbones and/or pendant groups and various  
20 degrees of sulfonation/functionalization. The ionized groups on  
21 these electrolyte matrices may include but are not limited to  
22 sulfonates, carboxylates, phosphates, and borates. Manipulation  
23 of the molecular weight and purity of the matrices allows for  
24 optimized polymerization and processing conditions.

1           The present invention further relates generally to a syn-  
2 enzymatic polymerization process of aniline in the presence of  
3 sulfonated multi wall carbon nano tubes (MWCNT) which results in a  
4 novel complex of polyaniline (pani) and MWCNT which has  
5 exceptional stability, and good processability.

6           Carbon nanotubes (CNT) have been the focus of extensive  
7 research since their discover. Several investigators have  
8 reported unique physical and mechanical properties for this form  
9 of carbon. CNT exhibit electronic properties with thermal  
10 conductivities higher than diamond and present significant  
11 opportunities for the development of novel multifunctional  
12 material systems. The mechanical properties of CNT, such as  
13 stiffness, strength and resilience, surpass those of most known  
14 materials. The combination of low density and high strength may  
15 also lead to the development of high performance nanotube  
16 reinforced composites. Nanotubes and/or nanowires have been shown  
17 to have application in electronic nanoscale devices, such as  
18 single-molecular transistors. Other potential applications are  
19 field emission electron emitters for flat panel displays, chemical  
20 sensors and artificial muscle actuators. There has been  
21 considerable scientific interest in the development of  
22 electrically conducting molecular wires involving these nanotubes.

23           Pani has been one of the most popular conducting polymers due  
24 to its low cost and excellent electronic and environmental  
25 stability. The applications of pani have been somewhat limited

1 due to poor processability/solubility. However, the use of  
2 polyelectrolytes as templates for the preferential alignment of  
3 monomer molecules, along with providing charge compensation  
4 (doping) in the polymer, has alleviated most of these problems.  
5 It has been demonstrated that templates, like polystyrene  
6 sulfonate, polyvinyl phosphonic acid, and biological templates,  
7 like DNA, can be used for the horseradish peroxidase (HRP)  
8 catalyzed synthesis of conducting water-soluble pani. Recently, a  
9 synthetic enzyme based on a hydroxy ferriprotoporphyrine hematin  
10 covalently bound to PEG, was found to be a cost-effective  
11 alternative to HRP. This synthetic enzyme, known as PEG-Hematin,  
12 was found to be more robust and allowed the flexibility to work  
13 over a broad range of pH, in contrast to HRP. It has been found  
14 that sulfonated CNT may be used as a template for the PEG-Hematin  
15 catalyzed polymerization of aniline. By doing so, one can produce  
16 nanowires comprised of conducting pani covering the CNT with  
17 dimensions on the order of tens of nanometers in diameter and  
18 microns in length.

19 The impetus behind the novel synthetic approach described  
20 herein is the use of an electrolyte matrix during the  
21 polymerization process. Here, the electrolyte serves three  
22 critical functions. First, the electrolyte (sulfonated MWCNT)  
23 serves as a matrix upon which the aniline monomers preferentially  
24 align to promote the head to tail coupling and extended  
25 conjugation of the resulting polymer chains. Secondly, the

1 electrolyte acts as a large molecular dopant species which is  
2 complexed and essentially "locked" to the pani chains. Current  
3 limitations to the actual use of pani and in electronic and  
4 optical applications have been due to poor dopant stability where  
5 the small ionic dopants or chromophores that are currently used  
6 are known to diffuse away with time and/or conditions and thus  
7 result in a loss of conductivity and/or optical activity. This  
8 "locking" of a large sulfonated MWCNT dopant to the pani chains is  
9 significant in that it ensures maintainment of the polymers  
10 "doped" form and hence stability of the desired electronic and  
11 optical properties. Lastly, the electrolyte template serves to  
12 promote mechanical stability of the final MWCNT/Pani complex.  
13 Since panis are known to be virtually unprocessable without harsh,  
14 chemical modification or involved synthetic strategies, this new  
15 approach provides unsurpassed environmentally compatible, facile,  
16 and inexpensive processing opportunities for real device  
17 fabrication.

18 The MWCNT sulfonate in these complexes allows for a wide  
19 range of applications including but not limited to nanowires in  
20 microchips, high performance nanotubes; reinforced conductive  
21 composites; single-molecular transistors, electron emitters for  
22 flat panel displays, chemical sensors and artificial muscle  
23 actuators.

24 The present invention is premised on the discovery that  
25 unsurpassed electrical and optical stability, processability,



1 tunability and environmental compatibility are imparted to a new  
2 matrix assisted syn-enzymatic polymerization of aniline and  
3 phenol. In addition, with judicious choice of matrix and/or  
4 monomer, the final polymer complex properties may be tailored to  
5 suit a wide range of real device applications.

6 The present invention will now be further described by the  
7 following non-limiting examples.

#### 8 9 EXAMPLE 1

#### 10 Synthesis of PEG-Hematin complex

11 The PEG-hematin complex was obtained through the coupling of  
12 PEG chains to a hematin molecule through ester linkages as shown  
13 in FIG. 1. The PEG-hematin complex was prepared by the addition  
14 of a mole equivalent of PEG (19 mg) to hematin (200 mg) in the  
15 presence of activators N, N'-carbonyldiimidazole (0.05 g) and 1,8-  
16 diazabicyclo[5.4.0]undec-7-ene (DBU) (0.047 g) in DMF. The  
17 mixture was allowed to stir for 48 hours then was quenched by the  
18 addition of a large volume of deionized water. The unreacted  
19 reagents were removed by extraction with ethyl acetate. The water  
20 layer was subsequently lyophilized to yield PEG-hematin as a  
21 reddish-brown solid.

22 The complex was characterized using NMR and FTIR  
23 spectroscopy. The average extent of modification of the acidic  
24 groups of hematin was determined using UV-vis spectroscopy. The  
25 UV-vis spectra of the PEG-hematin exhibited a decrease in the

1 Soret band (420 nm), a porphyrin centered  $\pi$ - $\pi^*$  transition, in  
2 comparison to hematin, which was used to calculate the amount of  
3 hematin present in the sample. However, the energy and spectral  
4 bandwidths of PEG-hematin were indistinguishable from hematin,  
5 which indicated that the modification of hematin by poly (ethylene  
6 glycol) does not affect the heme structure. Based on this  
7 assumption, the average concentration of hematin in the PEG-  
8 hematin sample was subsequently determined to be 67% by weight.

9 An FTIR spectrum of PEG-hematin indicated the presence of an  
10 ester functionality by the appearance of a doublet at 1646 and  
11 1651  $\text{cm}^{-1}$  (similar to diethyl phthalate) accompanied by the  
12 complete disappearance of the peak at 1712  $\text{cm}^{-1}$  for the acid  
13 carbonyl of hematin (FIG. 2). The strong peak at 1100  $\text{cm}^{-1}$   
14 corresponded to the ether linkage of the glycol moiety.

15 An  $^1\text{H}$  NMR spectrum of PEG-hematin in  $\text{DMF-d}_7$  shows the  
16 disappearance of the peak at 10.2 ppm, which was assigned to the  
17 carboxylic proton of hematin (FIG. 3a). This clearly indicated  
18 that the carboxylic acid hydroxyl moiety was transformed into an  
19 ester. The large broad peak at 3.8 ppm was assigned to the poly  
20 (ethylene glycol) protons. However, the spectra could not be well  
21 resolved in the region of 2-4 ppm due to the interference of the  
22 peaks assigned to the residual protons in deuterated DMF. In  
23 order to get a better resolution of the spectrum, the solvent  
24 system was changed to deuterated water. The spectrum  $\text{D}_2\text{O}$  could  
25 not be used to distinguish the absence of the carboxylic acid

1 proton due to proton exchange with D<sub>2</sub>O. However, comparison of  
2 the spectrum of PEG-hematin and spectrum of poly(ethylene glycol),  
3 in D<sub>2</sub>O showed the changes in the position of the PEG peaks of PEG-  
4 hematin in comparison to PEG alone. It was found the PEG  
5 exhibited a major peak at 3.8 ppm, which was assigned to the bulk  
6 of the polymer chains, while the adjoining peaks (triplets) were  
7 assigned to the end groups of the polymer. When a PEG-hematin  
8 derivative was formed, the peak at 4.0 ppm shifted upfield and  
9 merged into the main peak. This was accompanied by considerable  
10 broadening and a shift of the peak at 3.8 ppm to 3.6 ppm (FIG.  
11 3b). It was postulated that methylene protons  $\alpha$  to the hydroxy  
12 group PEG, on being attached by an ester linkage to hematin,  
13 shifted upfield while methylene protons  $\beta$  to the hydroxy groups of  
14 PEG were affected by the inhomogeneous paramagnetic environment,  
15 leading to broadening. These observed changes strongly indicated  
16 the formation of an ester bond between PEG and hematin.

17 The activity of the PEG-hematin was assessed through the  
18 oxidation of pyrogallo (0.5%) to purpurogallin in 14 mM potassium  
19 phosphate buffer in the presence of 0.027% (w/w) hydrogen  
20 peroxide. The activity of the PEG-hematin was found to be  
21 approximately 30-fold higher as compared to native hematin at a pH  
22 4.0 (FIG. 4). It is believed that the activity of hematin is  
23 dependent on its solubility. Thus, the enhanced activity of the  
24 PEG-hematin is attributed to its enhanced solubility.

## EXAMPLE 2

### Synthesis of Polyaniline

The polymerization of aniline was carried out in 0.1 M sodium phosphate buffer (10 mL) maintained at pH 1. To this buffer solution the aniline monomer was added. The catalyst, PEG-hematin (60  $\mu$ g), was added only just prior to the addition of hydrogen peroxide. The polymerization was initiated by the incremental addition of a stoichiometric amount of hydrogen peroxide, with respect to aniline. 0.3%  $\text{H}_2\text{O}_2$  (w/v) was used with constant stirring and the progress of the reaction was monitored spectroscopically (FIG. 5). Typically, all reaction systems were left stirred until completion of polymerization followed by precipitation of the pani. The pani synthesized was filtered off and thoroughly washed with acetone a few times followed by drying in a vacuum oven. The conductivity of the pani pellet was found to be of the order of 0.2 S/cm.

This reaction thus proved the versatility and ability of the PEG-Hematin for the synthesis of stable conducting pani even in the absence of template. The pani formed in this case was again redox reversible as proved by cyclic voltammetry studies.

## EXAMPLE 3

### Synthesis of Sodium Poly (sodium-4-styrenesulfonate)-Polyaniline Complex

1           The polymerization of aniline was carried out in 0.1 M sodium  
2 phosphate buffer over a range of pH conditions from pH 1-4. A 17  
3 mM solution of SPS template in phosphate buffer (100mM) was  
4 prepared to which the aniline monomer was added in a 1:1 molar  
5 ratio of aniline to sodium styrene sulfonate monomer. The  
6 catalyst, PEG-hematin (5 mg), was added just prior to the addition  
7 of hydrogen peroxide. The polymerization was initiated by the  
8 incremental addition of a stoichiometric amount of hydrogen  
9 peroxide (relative to aniline). In all cases, 0.3% H<sub>2</sub>O<sub>2</sub> (w/v) was  
10 used with constant stirring, and the progress of the reaction was  
11 monitored spectroscopically. On completion of polymerization, the  
12 solution was transferred to individual regenerated natural  
13 cellulose membrane bags (molecular weight cut-off 10,000 D) and  
14 were dialyzed against 5000 mL of acidified deionized water  
15 maintained at pH 4.0 to remove unreacted monomers and oligomers.  
16 The solid SPS-Pani complex was obtained by evaporation of the  
17 deionized water followed by drying in a vacuum oven.

18           It was observed that the solution slowly turned dark green,  
19 indicating the formation of the doped emeraldine salt form of  
20 conducting pani. The UV-vis absorption spectra of the Pani/SPS  
21 complex, formed at different time intervals over a period of 2  
22 hours at pH 4.0 after initiation of polymerization reaction, is  
23 shown in FIG. 6. The UV-vis spectra showed the presence of  
24 polaron absorption bands at 400 nm and 800-1200 nm, which was  
25 consistent with the formation of the conducting form of pani.

1 This polymerization was also carried out at different pH values  
2 ranging from pH 1.0 to pH 4.0 as shown in FIG. 7. The formation  
3 of pani was observed in all cases, thus demonstrating the  
4 stability and robustness of the PEG-hematin in comparison to  
5 hematin (insoluble at low pH) or HRP (denatured at low pH). Also,  
6 the pani formation reaction catalyzed by PEG-hematin was found to  
7 be complete with greater than 90% yield within a few hours, while  
8 the unmodified hematin showed little or no reactivity within the  
9 same time period under these acidic conditions.

10 The redox tunability of the pani formed was further  
11 demonstrated by dedoping the emeraldine salt form of pani at high  
12 pH and then redoping with acid. With increasing pH (dedoping) on  
13 titration with 1 N NaOH, the polaron bands at 400 nm and 800 nm  
14 were found to diminish, while a new band at 600 nm began to emerge  
15 due to the exciton transition of the quinoid ring giving rise to a  
16 blue solution indicating that the Pani had been fully dedoped to  
17 the base form. On titrating the solution back with 1 N HCl  
18 (redoping), a reversible color change was observed and the spectra  
19 is shown in FIG. 8. Furthermore, an isosbestic point at 710 nm  
20 was also observed, which was indicative of the changes in the pani  
21 oxidation state. This behavior was similar to the pani  
22 synthesized chemically or enzymatically with HRP and confirmed the  
23 formation of the conducting pani emeraldine salt form  
24 (electroactive form) catalyzed by PEG-hematin.

1           The conductivity of the emeraldine salt form of pani  
2 synthesized at pH less than 4 was found to be about  $10^{-3}$  S/cm.

3           Furthermore, cyclic voltammetry studies were carried out to  
4 determine the electrochemical nature of pani synthesized by the  
5 PEG-hematin catalysis. The cyclic voltammogram of a cast film of  
6 an SPS-pani complex (FIG. 9) showed two sets of peaks indicating  
7 two reversible redox cycles at a scan rate of 100 mV/s over a  
8 potential window of -0.2-1.2V.

#### 10       EXAMPLE 4

##### 11       Synthesis of Lignosulfonate-Pani Complex

12           5.2 mg of a lignin sulfonate polyelectrolyte complex was  
13 dissolved in 10 mL of sodium monophosphate buffer (0.1 M)  
14 maintained at pH 4.0. This was followed by the addition of 18  $\mu$ L  
15 of aniline, a catalytic amount of PEG-Hematin and an amount of  
16 hydrogen peroxide (0.3%) stoichiometric with aniline. The  
17 reaction mixture was allowed to stir until precipitation of the  
18 polyelectrolyte-Pani complex ceased. The reaction was also  
19 carried out in solutions having pHs ranging from pH 1-4 (FIG. 10).  
20 The precipitated lignin sulfonate-Pani complex obtained was washed  
21 several times with acidified acetone to remove the unreacted  
22 monomer and finally washed with acidified deionized water,  
23 filtered under suction through a polycarbonate filter and dried in  
24 a vacuum oven to yield lignin sulfonate-polyaniline complex.

1           When the polymerization was conducted at pH 3.0, there was a  
2       peak of low intensity at 767 nm for the emeraldine form of  
3       polyaniline, which was completely absent during polymerization at  
4       pH 4.0. The extended absorption of 1200 nm indicated the  
5       formation of the extended conjugation of the pani backbone. Thus,  
6       the synthesis of pani complexed with a natural polymer further  
7       widens the scope of applications to other natural polyelectrolytes  
8       to form versatile, environmentally benign conducting polymers.

#### 10       EXAMPLE 5

##### 11       Synthesis of DNA-Pani Complex

12           The polymerization of aniline in the presence of Calf Thymus  
13       DNA was carried out in sterile 10 mM phosphate buffer. A 1.0 mM  
14       calf thymus DNA solution was prepared by dissolving the required  
15       amount of DNA in 10 mL of sterilized sodium phosphate buffer  
16       maintained at pH 4. The concentration of DNA was determined by  
17       the UV absorbance at 258 nm. To this DNA solution, 4.5  $\mu$ l (5 mM)  
18       of aniline was added. The pH of the solution was again checked  
19       and adjusted to 4.3, and 5 mg of PEG-Hematin were added. To this  
20       reaction mixture, a solution of hydrogen peroxide (0.3% solution,  
21       4.5  $\mu$ l, 5 mM) was added drop-wise, to initiate the polymerization  
22       and reaction of aniline was followed using UV-Vis spectroscopy and  
23       circular dichroism polarimetry.

24           When the aniline monomer was added to a DNA solution at pH  
25       4.3, the electrostatic interaction between the protonated aniline



1 monomers and the phosphate groups in the DNA caused the monomers  
2 to closely associate with the DNA. The association of the  
3 protonated aniline monomer on the DNA template facilitated a  
4 predominantly para-directed coupling and inhibited parasitic  
5 branching during the polymerization. The high proton  
6 concentration around the phosphate groups also provided a unique  
7 local lower pH environment that permitted the polymerization of  
8 aniline at a higher pH than that necessary with conventional  
9 chemical polymerization of aniline. The polymerization was  
10 catalyzed by PEG-hematin and initiated by hydrogen peroxide.  
11 However, as the polymerization proceeded over a period of time and  
12 a critical chain length was attained, the DNA-Pani complex  
13 precipitated out of solution. It was concluded that the complex  
14 remained soluble as long as there were enough phosphate groups on  
15 the DNA available for solvation. As the polymerization proceeded,  
16 the preferred molecular interaction between the charged aniline  
17 groups and the phosphate groups of DNA caused the growing chain to  
18 occupy a majority of these sites leading to the salting out of the  
19 DNA-Pani complex. The polymerization reaction was followed using  
20 UV-vis spectroscopy and circular dichroism polarimetry. The UV-  
21 vis spectra of the DNA-Pani complex recorded after initiation of  
22 the polymerization are shown in FIG 11. The UV-vis absorbance  
23 spectra showed a peak around 260 nm emerging from the absorption  
24 of the base pairs of DNA along with polaron absorption bands at

1 420 nm and 750 nm, indicating the formation of the conducting  
2 emeraldine salt form of polyaniline.

3 The bases of the nucleic acid have a plane of symmetry and  
4 thus are not intrinsically optically active. However, the  
5 deoxyribose sugar is asymmetric and since the bases are attached  
6 to the anomeric carbon of these sugars, the sugar can induce a  
7 circular dichroism in the absorption bands of the bases. These  
8 bands may be observed either for the intensely electronically  
9 allowed  $\pi$ - $\pi^*$  transitions, or for the weakly allowed  $n$ - $\pi^*$   
10 transitions because these transitions are magnetically allowed.  
11 Also, the  $\pi$  electron systems of the bases make them hydrophobic,  
12 so the bases tend to stack in hydrogen-bonding solvents to  
13 minimize the  $\pi$ -electron surface area exposed to the solvent. The  
14 hydrophobic planes and hydrophilic edges as well as charge-charge  
15 interactions cause the bases to stack and the polymer to adopt a  
16 helical structure. Preferential handedness is induced in these  
17 helical structures by the intrinsically asymmetric sugars, giving  
18 the DNA polymer a whole super asymmetry. The electronic  
19 transitions of these chromophoric bases are in close proximity and  
20 can thus interact to give well-defined CD spectra. The CD  
21 spectrum of the DNA-Pani complex showed a reduction in the  
22 intensity of the peak at 275 nm (FIG. 12). This change indicated  
23 a polymorphic transition in DNA causing the DNA to change from a  
24 loosely wound form to the over-wound form. The appearance of a  
25 positive peak at 450 nm indicated that the helical polyelectrolyte

1 DNA template induces a macroscopic order in the pani that is  
2 formed. This result proves the extensive versatility of the PEG-  
3 Hematin catalyst with a variety of templates, including delicate  
4 biomacromolecules, in providing the optimal catalytic activity for  
5 polymerization.

#### 6 7 EXAMPLE 6

#### 8 Synthesis of Poly(2-methoxy, 5-methylaniline)-SPS complex

9 The polymerization of 2-methoxy, 5-methylaniline (2M5M) was  
10 carried out in 0.1 M sodium phosphate buffer of pH 4.0. A 17 mM  
11 SPS template solution, as measured from the concentration of  
12 sodium styrene sulfonate monomers, in phosphate buffer (10 mL) was  
13 prepared, to which 2M5M (24 mg) was added in the desired (1:1,  
14 2M5M:SPS) molar ratio. The polymerization was initiated after  
15 addition of 5 mg of PEG-Hematin, by the incremental addition of an  
16 amount of peroxide (0.3% w/v) stoichiometric with 2M5M, with  
17 constant stirring. The progress of the reaction was monitored  
18 spectroscopically. After the reaction was complete, the solution  
19 was dialyzed to remove the unreacted monomers, followed by  
20 evaporation to yield a SPS-poly(2M5M) complex.

21 The UV-vis absorption spectra of the poly(2M5M)/SPS complex  
22 formed is shown in FIG. 14. The spectra again showed the presence  
23 of a polaron band at 425 nm and extended conjugation in the longer  
24 wavelength range indicating the linear conducting form of pani.  
25 This polymer also showed reversible redox tunability similar to

1 that observed for the SPS-pani complex formed in Example 2. The  
2 SPS-poly(2M5M) formed could also be reversibly de-doped on  
3 titrating with 1N NaOH and re-doped by back titrating with 1N HCL.  
4

#### 5 EXAMPLE 7

##### 6 Synthesis of Sodium Dodecylbenzenesulfonic Acid-Pani Complex

7 Polymerization of aniline was carried out in 0.1 M sodium at  
8 pH 4. At 17 mM solution of dodecylbenzenesulfonic acid (DBSA) in  
9 phosphate buffer (100 mM) was prepared to which the aniline  
10 monomer was added in the desired (1:1, Aniline:DBSA) molar ratio.  
11 The catalyst, PEG-Hematin (5 mg), was added just prior to the  
12 addition of hydrogen peroxide. The polymerization was initiated  
13 by the incremental addition of an amount of hydrogen peroxide  
14 stoichiometric to aniline. In all cases, 0.3% H<sub>2</sub>O<sub>2</sub> (w/v) was used  
15 with constant stirring. The progress of the reaction was  
16 monitored spectroscopically.  
17

#### 18 EXAMPLE 8

##### 19 Synthesis of SPS-Polyphenol Complex

20 A polymerization reaction was carried out in 10 mL of aqueous  
21 phosphate buffer (100 mM). The pH of the reaction media for the  
22 phenol polymerization was maintained at pH 7.0 and equimolar  
23 concentrations (17 mM) of SPS, with respect to the concentration  
24 of the repeat units, and phenol monomer were added to the buffered  
25 solution, followed by 10 mg of the PEG-hematin. The reaction was

1 initiated by addition of a stoichiometric, with respect to phenol,  
2 amount H<sub>2</sub>O<sub>2</sub> (30% w/v) in one lot to facilitate the formation of  
3 high molecular weight polypenol. The reaction was monitored  
4 spectroscopically. A control experiment was also carried out  
5 simultaneously in the absence of catalyst. The final products  
6 were dialyzed using Centricon concentrators (10,000 Mw cut off,  
7 Amicon Inc., Beverly, MA) to remove unreacted monomers. The  
8 samples were then dried under vacuum at 50°C and used for further  
9 analysis. The yield was calculated to be typically 95% or higher.

10 The PEG-hematin complex was also found to catalyze the  
11 polymerization of phenol at pH 7.0 more efficiently than that  
12 compared to the native hematin and peroxidase (FIG. 15). The  
13 large broad absorption tail in the region from 300-700 nm  
14 conferred the presence of extended conjugation and indicated  
15 formation of polyphenol by PEG-hematin reaction. In comparison,  
16 the absorption of the hematin-catalyzed reaction was relatively  
17 weak. Thus, modification of the hematin with PEG was observed to  
18 significantly improve the reactivity to suit the desired reaction  
19 conditions leading to the formation of polyphenol.

## 20 21 EXAMPLE 9

### 22 Preparation of Assembled Hematin

23 Glass slides (25 by 75 mm) were hydrophilized with 1% Chem-  
24 solv solution in deionized water under ultrasonication for use as  
25 substrates. This treatment generates negative charges on the

1 surface of the slides due to partial hydrolysis. After 3 hours,  
2 the slides were ultrasonicated twice in deionized water for 30  
3 minutes before use.

4 The electrostatic layer-by-layer deposition process was  
5 carried out in two steps. PDAC (10 mM) and hematin (3 mM)  
6 solutions were prepared over a pH range from 5 to 11. In the  
7 first step, hydrophilized glass slides were immersed in PDAC  
8 solution for 10 minute at room temperature and washed with  
9 deionized water for 5 minutes. After the deposition and washing  
10 steps, the slides were dried with a stream of nitrogen. In the  
11 second step, the substrates with a single layer of PDAC were  
12 immersed into the hematin solution for 10 minutes and subsequently  
13 washed with deionized water and dried with a stream of nitrogen to  
14 produce an assembled hematin, having a bilayer film of  
15 PDAC/hematin. This dipping procedure was iterated to build up  
16 multilayer films.

#### 18 EXAMPLE 10

##### 19 Synthesis of Pani-SPS Complex Using Assembled Hematin

20 Polymerization of aniline was carried out at room temperature  
21 in a 40mL, 0.1M phosphoric acid buffer solution, which contained a  
22 1:1 molar ratio of SPS (MW 1,000,000; moles correspond to quantity  
23 of monomers units) to aniline 0.167g (0.81mmol). SPS was added  
24 first to the buffered solution, followed by an addition of 2.1mL  
25 of aniline stock solution (0.036mL aniline to 1 mL buffer at pH

1 1.4) with constant stirring. A seventeen bilayer Hematin/PDAC  
2 assembly was immersed in the solution. To initiate aniline  
3 polymerization, 11mL of 0.25% H<sub>2</sub>O<sub>2</sub> was added dropwise,  
4 incrementally, over 30 minutes. The reaction was maintained for  
5 24 hours, and carried out at different pH values (1.0, 2.0, 3.0).  
6 The rate of assembled hematin catalyzed polymerization was  
7 monitored by a Perkin-Elmer Lambda-9-UV-vis spectrophotometer at  
8 room temperature.

#### 10 EXAMPLE 11

##### 11 Synthesis of Aniline Monomer in Presence of MWCNT

12 Aniline monomer polymerizes in the presence of sulfonated  
13 multiwall carbon nano tubes (MWCNT), an oxidizing agent which is  
14 comprised of syn-enzyme (Hematin-PEG) and an electron acceptor  
15 (hydrogen peroxide) to give a MWCNT sulfonate/pani complex which  
16 is dispersed in water. The pani as synthesized may be  
17 simultaneously oxidized at higher pH's than can be done using  
18 prior art techniques, resulting in the emeraldine "conducting"  
19 form and undergoes reversible oxidation and reduction with change  
20 of pH.

#### 22 EXAMPLE 12

##### 23 Synthesis of 2-Methoxy, 5-Methylaniline in presence of MWCNT

24 2-methoxy, 5 methylaniline polymerizes in the presence of  
25 sulfonated MWCNT, an oxidizing agent which is comprised of syn-

1 enzyme (Hematin-PEG) and an electron acceptor (hydrogen peroxide)  
2 to give a MWCNT sulfonate/pani complex which is dispersed in  
3 water. The pani as synthesized may be simultaneously oxidized at  
4 significantly higher pH's than can be done using prior art  
5 techniques, resulting in the emeraldine "conducting" form and  
6 undergoes reversible oxidation and reduction with change of pH.  
7

#### 8 EXAMPLE 13

#### 9 Synthesis of Phenol Monomer in Presence of MWCNT

10 Phenol monomer polymerizes in the presence of sulfonated  
11 MWCNT, an oxidizing agent which is comprised of syn-enzyme  
12 (Hematin-PEG) and an electron acceptor (Hydrogen peroxide) to give  
13 a MWCNT sulfonate/polyphenol complex which is dispersed in water.  
14 This polyphenol complex may be an environmentally friendly, cost  
15 effective, and more processable substitute for current  
16 polyphenolic resin materials.  
17

18 This invention provides a significant advancement over  
19 current methods used for the synthesis of a conducting form and  
20 processable form of polyaniline. This approach addresses and  
21 resolves processability and stability of the current limitations  
22 of the commercial use of polyaniline. The syn-enzymatic synthesis  
23 provides a specific, simple (one-step) and environmentally  
24 friendly synthetic approach, while the MWCNT provides mechanical  
25 stability and processability. In addition, since MWCNT shows



1 extremely interesting applications in liquid display and already  
2 used for commercial applications, the MWCNT/polyaniline complex  
3 described in this invention is expected to transition effectively  
4 into many of these already established applications where  
5 electrical activity and/or conductivity is desirable. Examples of  
6 such applications include chemical and biological sensing,  
7 electrostatic shielding, corrosion protection, light weight  
8 rechargeable batteries, flexible light-emitting diodes,  
9 electrochromic devices, smart windows, chaff materials, radiation  
10 absorbers for optical illusions, nanowires in microchips, high  
11 performance nanotube; reinforced conductive composites; single-  
12 molecular transistors, electron emitters for flat panel displays,  
13 chemical sensors and artificial muscle actuators.

14 While this invention has been particularly shown and  
15 described with references to preferred embodiments thereof, it  
16 will be understood by those skilled in the art that various  
17 changes in form and details may be made therein without departing  
18 from the scope of the invention encompassed by the appended  
19 claims.